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PANCREATIC ACINAR CELLS: MEASUREMENT OF MEMBRANE POTENTIAL AND MINIATURE DEPOLARIZATION POTENTIALS

BY P. M. DEAN AND E. K. MATTHEWS

From the Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 3EF

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SUMMARY

1. Intracellular recordings of membrane potentials have been made *in vitro* from the exocrine acinar cells of the mouse pancreas using glass microelectrodes.

2. The mean membrane potential of the acinar cells was $-41{\cdot}2$ mV. Spontaneous miniature depolarization potentials of 0·5–5·0 mV amplitude and occurring at random intervals were often observed superimposed upon the resting membrane potential. Atropine $2{\cdot}8 \times 10^{-6}$ M decreased, and physostigmine $1{\cdot}23 \times 10^{-5}$ M increased, the frequency and amplitude of the miniature potentials.

3. Electrical stimulation of the pancreatic nerves depolarized the acinar cells. Acetyl β-methylcholine 10^{-6} M produced a sustained depolarization. Atropine $1{\cdot}4 \times 10^{-6}$ M blocked the depolarization to both electrical stimulation and acetyl β-methylcholine.

4. Pancreozymin depolarized the exocrine acinar cells but secretin did not.

5. These results suggest that the miniature depolarization potentials are of cholinergic origin and that depolarization of the pancreatic exocrine cells by nerve stimulation or pancreozymin may trigger secretion.

INTRODUCTION

Enzyme secretion from the exocrine pancreas is controlled largely by the hormone pancreozymin and by cholinergic innervation to the pancreatic acinar cells through branches of the vagal nerves (Harper & Raper, 1943; Grossmann, 1962). Recent studies from many types of secretory cells have drawn attention to the importance of the plasma membrane in the secretory mechanism. Changes in the electro-physiological properties

of the membrane of adrenal medullary cells (Douglas, Kanno & Sampson, 1967), β-cells from pancreatic islets (Dean & Matthews, 1970*a*, *b*) and salivary glands (Lundberg, 1955), are concomitant with the release of secretory product. Although much is known about the biochemical and secretory properties of the exocrine pancreas, the acinar cells have not previously been the subject of electrophysiological study. In the experiments described in this paper we have measured the membrane potential of acinar cells from mouse pancreas and studied the effects of some parasympathomimetic agents and the hormones pancreozymin and secretin upon the membrane potential. A preliminary account of this work has already been given (Dean & Matthews, 1968).

METHODS

The pancreas was removed from albino mice of either sex weighing 30–40 g. Segments of pancreas were secured to a small Perspex platform placed in a Perspex tissue bath and exposed to a continuous flow of Krebs–Henseleit solution warmed to 37° C. The solution had the following composition: NaCl 103 mM, KCl 4·7 mM, $CaCl_2$ 2·56 mM, $MgCl_2$ 1·13 mM, $NaHCO_3$ 25 mM, NaH_2PO_4 1·15 mM, D-glucose 2·8 mM, sodium pyruvate 4·9 mM, sodium fumarate 2·7 mM, sodium glutamate 4·9 mM; it was gassed with 95% O_2 and 5% CO_2. The connective tissue sheath overlying the lobes of acinar tissue was removed by microdissection. This was facilitated by transverse illumination and use of a Zeiss stereomicroscope (× 40).

Glass micro-electrodes filled with 1·5 M potassium citrate and having a tip resistance of about 100 MΩ were used for recording cellular transmembrane potentials according to the method of Dean & Matthews (1970*a*). The criterion for an acceptable measurement of membrane potential was that the potential should appear suddenly, be maintained for at least 1 min and return instantly to zero potential as the electrode was withdrawn from the cell. The tissue was allowed to equilibrate in Krebs–Henseleit solution for 40 min before the membrane potentials were recorded.

The vagus nerve enters the pancreas through the perivascular sheath of the pancreatico-duodenal artery (Rickins, 1945) and could not be easily separated from this sheath without damage. In the experiments where the pancreatic nerve was stimulated, the whole perivascular sheath, including nerve, artery and veins, was carefully exposed by microdissection. A small bipolar silver electrode was hooked under the nerve-perivascular sheath complex and carefully lifted above the bathing solution during stimulation. The stimulus was delivered from a Grass S 4 stimulator and the stimulus was isolated from ground to minimize the stimulation artifact. Miniature and membrane potentials were recorded from the oscilloscope on photographic film and on paper by a Devices M 2 pen recorder.

RESULTS

The distribution of acinar cell membrane potentials is shown in Fig. 1. The mean value is $-41{\cdot}2 \pm$ S.E. of mean 1·4 mV. Membrane potentials were well maintained throughout an experiment (in some instances up to 8 hr) and single impalements of acinar cells could often be maintained for

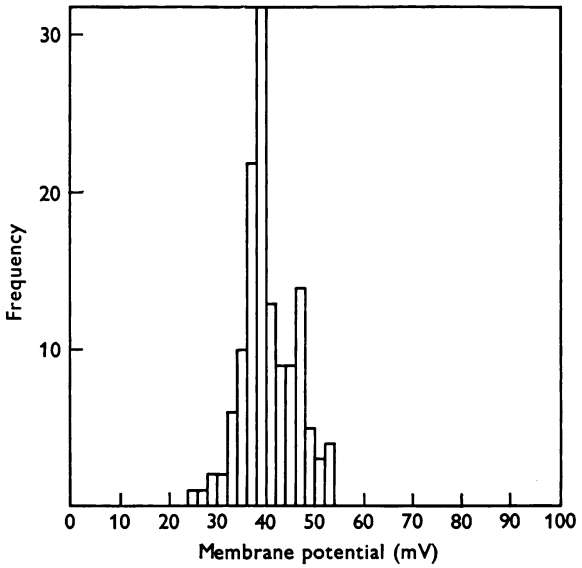

Fig. 1. Frequency distribution of membrane potentials recorded from the acinar tissue of mouse pancreas.

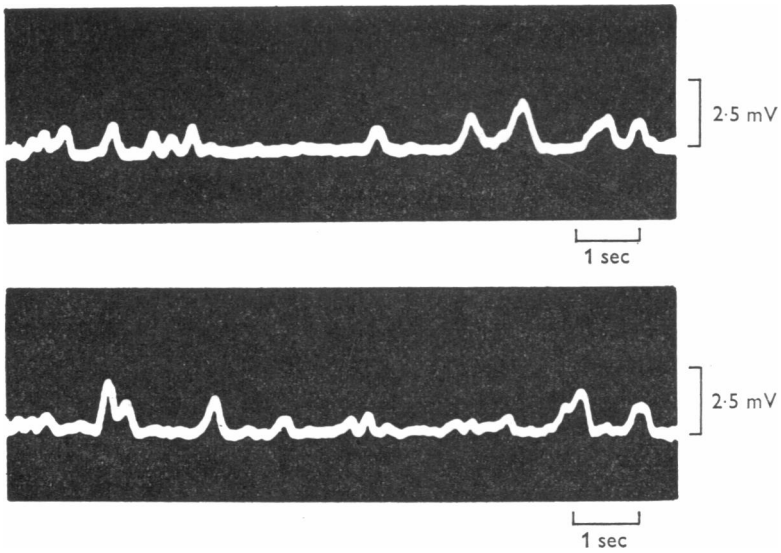

Fig. 2. Miniature depolarization potentials superimposed on the resting potential of pancreatic acinar cells.

up to 1 hr. Spontaneous miniature depolarization potentials were frequently observed superimposed on the resting potential of the acinar cells (Fig. 2). These small potential changes resembled the miniature end-plate potentials found at the neuromuscular junction (Fatt & Katz, 1952). The amplitude of the miniature potentials varied from 0·5 to 5 mV with a duration of about 100 msec. The distribution of the amplitude of the miniature potentials showed a positive skew (Fig. 3*A*). The discharges occurred at random intervals and the frequency distribution for the time interval between successive discharges also displayed a positive skew (Fig. 3*B*).

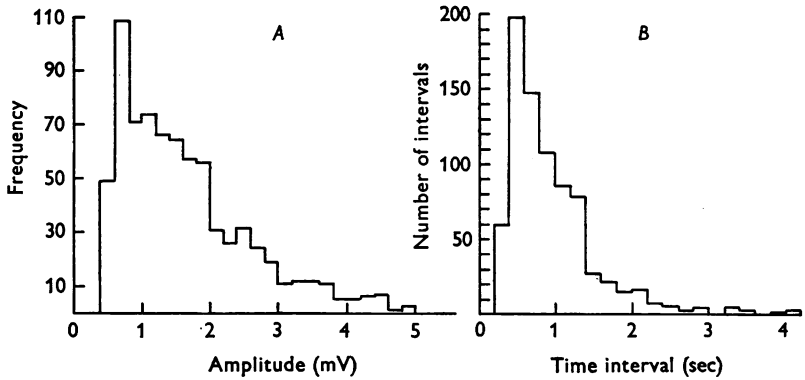

Fig. 3*A*. The frequency distribution for the amplitudes of the miniature depolarization potentials from an acinar cell (762 miniature potentials, intervals 0·2 mV).

B, the frequency distribution of the time intervals between the miniature depolarization potentials; time interval 0·2 sec.

The apparent random nature of the discharge was analysed statistically according to the method of Fatt & Katz (1952). In a random discharge the probability of the occurrence of a discharge is independent of the preceding activity; for an interval δt, very brief compared with the mean interval T, the probability of at least one discharge is given by $P = \delta t/T$. For a larger interval t, the probability of discharge increases exponentially and is calculated from the equation:

$$P = 1-\exp(-t/T).$$

This relationship was tested on a series of 762 miniature potentials from one cell covering a total period of 651 sec with a mean rate of 1·17 sec^{-1} and a mean interval of 0·86 sec. Plotting the total number of intervals of duration less than t against the interval duration t gave an exponential curve similar but not identical to the predicted curve for a stochastic

TABLE 1. Time series analysis of miniature depolarization potentials in pancreatic acinar cells

Expt no.	Mean frequency (m)		Interval probability (r)						No. periods	χ^2
1	2·88	r	⩽ 1	2	3	4	5	⩾ 6	68	$P < 0{\cdot}2$
		N_0	15	12	18	13	3	7		
		N_T	14·8	15·8	15·2	10·9	6·3	4·2		
2	3·28	r	⩽ 1	2	3	4	5	⩾ 6	58	$P < 0{\cdot}05$
		N_0	7	12	10	19	6	4		
		N_T	9·3	11·7	12·8	10·6	6·9	5·6		
3	7·4	r	⩽ 5	6	7	8	9	⩾ 10	50	$P < 0{\cdot}2$
		N_0	9	8	11	6	9	7		
		N_T	11·6	7·0	7·4	6·8	5·6	10·8		
4	9·56	r	⩽ 8	9	10	11	12	⩾ 13	71	$P < 0{\cdot}2$
		N_0	21	17	13	9	4	7		
		N_T	27·8	13·1	8·7	7·6	6·0	7·8		

process (Fig. 4). Therefore as a further test for the Poissonian character of the pancreatic miniature potential discharge the data of this and other experiments were subjected to a time series analysis similar to that described by Gage & Hubbard (1965) for miniature end-plate potentials. The probability of a unit time interval containing $r = 0, 1, 2, \ldots n$ miniature depolarization potentials was derived from the Poisson formula

$$p(r) = e^{-m} \cdot \frac{m^r}{r!}$$

and the closeness of fit between the observed (N_0) and theoretical (N_T) frequencies was determined by the χ^2 test with $n-2$ degrees of freedom (Table 1). In all four experiments (including Expt no. 4, that from which

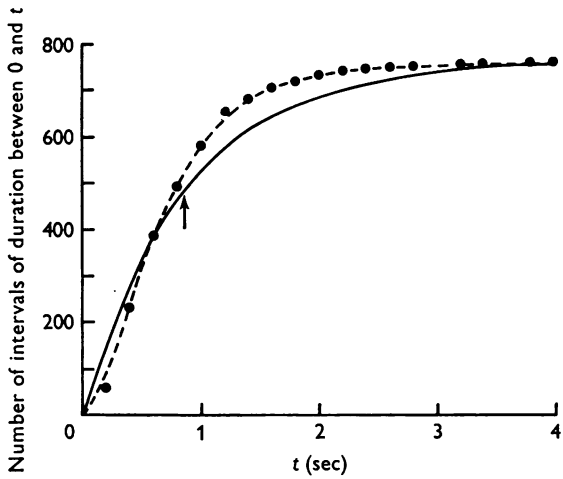

Fig. 4. The number of intervals of duration between 0 and t plotted against the interval duration t. Interrupted line: observed points. Continuous line: the theoretical curve drawn according to the equation

$$y = N(1-\exp[-t/T]),$$

where N is the total number of observations and T the mean time interval (indicated by the arrow).

Fig. 4 is derived) the χ^2 value obtained from a comparison between the observed and theoretical interval distributions was equivalent to $P < 5$–20 %, indicating that on the null hypothesis the results are not inconsistent with a Poisson distribution.

The action of drugs which affect cholinergic transmission

Exposure of acinar cells to Krebs–Henseleit solution containing acetyl β-methylcholine 10^{-6} M depolarized the acinar cells from a control value

of $-38{\cdot}9 \pm 2{\cdot}4$ mV to $25{\cdot}2 \pm 2{\cdot}1$ mV (Fig. 5); the depolarization was significant ($P < 0{\cdot}05$). The miniature potentials, which were observed during the control period, disappeared when the cells were depolarized by acetyl β-methyl choline 10^{-6} M. Superfusion with atropine $1{\cdot}4 \times 10^{-6}$ M 10 min before and during exposure to acetyl β-methylcholine 10^{-6} M prevented acinar cell depolarization. The membrane potential during the control period was $-38{\cdot}9 \pm$ S.E. of mean 2·5 mV and during the 30–60 min period

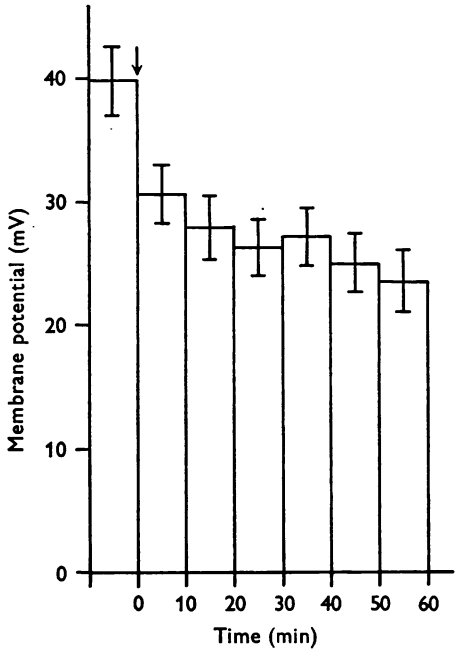

Fig. 5. The effect of acetyl β-methylcholine on the membrane potential of acinar cells. The first column represents the membrane potential measured during the 60 min control period. Acetyl β-methylcholine (10^{-6} M) was added at the arrow. The membrane potentials are the mean values $\pm$ S.E. of mean recorded from five preparations.

after acetyl β-methylcholine in the presence of atropine it was $-42{\cdot}2 \pm$ S.E. of mean 1·7 mV.

Fig. 6 shows a recording from a single acinar cell. During the control period in normal Krebs–Henseleit solution numerous miniature potentials were observed. When the tissue was superfused with a solution containing atropine $2{\cdot}8 \times 10^{-6}$ M the miniature potentials disappeared. In all five experiments in which it was tested atropine $2{\cdot}8 \times 10^{-6}$ M rapidly and completely blocked the miniature potentials.

Superfusion of the acinar tissue with the anticholinesterase agent

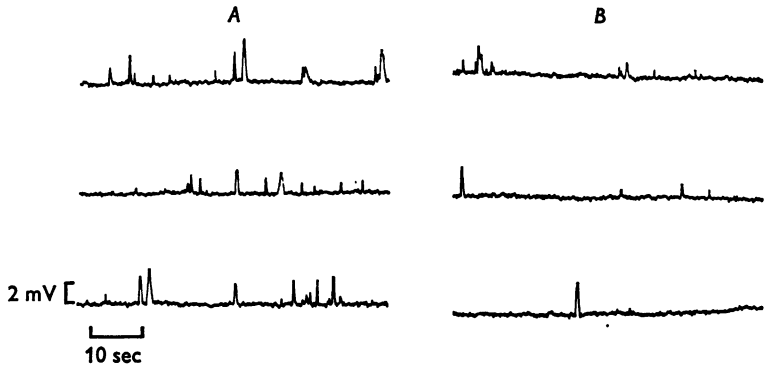

Fig. 6. The effect of atropine on the miniature depolarization potentials recorded from a single acinar cell. Miniature depolarization potentials occurred frequently during the control period (*A*). Thirty seconds after the addition of atropine $2{\cdot}8 \times 10^{-6}$ M the miniature potentials were progressively blocked (*B*). Each trace in *A* and in *B* is a consecutive recording.

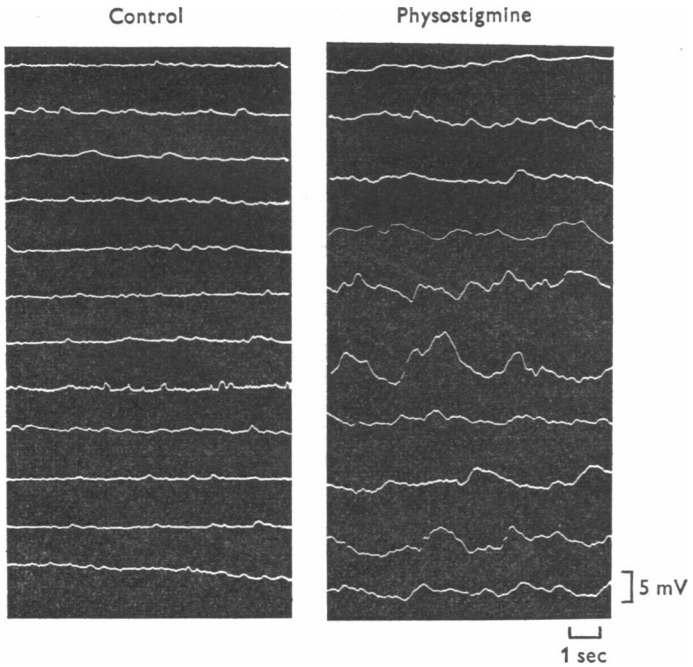

Fig. 7. The effect of the anticholinesterase drug physostigmine on the miniature potentials of acinar cells. Each record shows the membrane potential from successively impaled acinar cells. Small miniature potentials were observed during the control period; after the addition of physostigmine $1{\cdot}2 \times 10^{-5}$ M large fluctuations in the membrane potential occurred.

physostigmine $1{\cdot}2 \times 10^{-5}$ M did not affect the membrane potential but did enhance the spontaneous electrical activity. Fig. 7 shows the membrane potential of successively impaled cells. During the control period miniature potentials of 1–2 mV occurred. After the introduction of physostigmine large fluctuations of up to 5 mV in the membrane potential were observed with slow waves of up to 2 sec duration.

Electrical stimulation of the pancreatic nerve

The perivascular pancreatic vagus nerve was stimulated at a rate of 10 Hz with pulses of 2 msec duration and 7 V amplitude; this produced after a short delay of about 1 sec a depolarization of 10–15 mV in an impaled acinar cell. The cell repolarized when the stimulation ceased (Fig. 8*A*).

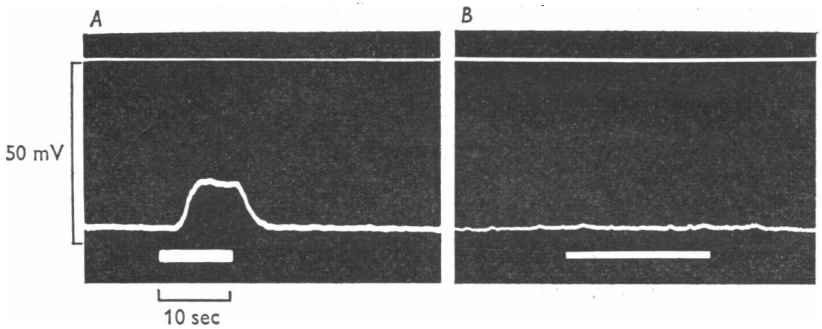

Fig. 8*A*. Depolarization of an acinar cell produced by stimulating the perivascular pancreatic nerve for 10 sec at 10 Hz, pulse duration 2 msec and amplitude 7 V (at the white bar).

B, superfusion with solution containing atropine $1{\cdot}4 \times 10^{-6}$ M prevented the same acinar cell from depolarizing when the perivascular pancreatic nerve was stimulated for 20 sec (at the white bar).

Superfusion with atropine $1{\cdot}4 \times 10^{-6}$ M 5 min before electrical stimulation prevented depolarization of the cell when the nerve was restimulated (Fig. 8*B*).

Effect of pancreozymin and secretin

When the acinar cells were superfused with solution containing the hormone pancreozymin 0·32 u./ml. (Crick, Harper & Raper Units) they rapidly depolarized from $-39{\cdot}4 \pm 2{\cdot}5$ mV to $-24{\cdot}1 \pm 2{\cdot}1$ mV, measured in the period 30–60 min after exposure to pancreozymin (Fig. 9). The depolarization was significant ($P < 0{\cdot}05$). This concentration of pancreozymin did not initially affect the frequency or the amplitude of the miniature potentials. However, once the cells had depolarized miniature potentials were no longer observed. In contrast to the action of pancreozymin, secretin 0·15 u./ml. had no effect on the acinar cell membrane potentials Fig. 9).

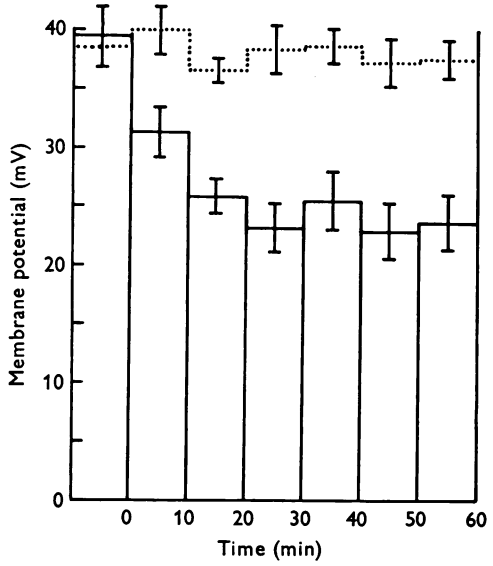

Fig. 9. The effects of pancreozymin and secretin on acinar cell membrane potentials. The first column shows the mean membrane potential obtained during the 60 min control period. Superfusion with pancreozymin 0·32 u./ml. beginning at 0 min depolarized the acinar cells (full histogram). The effect of secretin 0·15 u./ml. is shown in the stippled histogram.

DISCUSSION

In common with many secretory cells the mouse pancreatic acinar and islet cells have a low membrane potential relative to nerve and muscle, that is, $-41{\cdot}2$ mV for acinar cells and $-20{\cdot}1$ mV for islet cells (Dean & Matthews, 1970*a*). The reason for the low membrane potentials of these secretory cells is not clear. The possibility cannot be ruled out that the basal secretion of protein causes some membrane disruption, a consequent ionic flux and a reduction of the pancreatic cell membrane potential. More probably, however, the low membrane potential results from a higher Na^+ to K^+ permeability ratio which may exist in pancreatic and other cells (Matthews, 1967) compared with the ratio for nerve and muscle cells (Williams, 1970).

The discovery of miniature depolarization potentials in the acinar cells gives rise to questions about their origin; are they cholinergic like the miniature end-plate potentials at the neuromuscular junction, and like them due to the quantal release of acetylcholine from the nerve endings, or are they due to the actual discharge of secretory granules from the acinar

cells? Stimulation of the pancreatic nerve, a branch of the vagus, depolarized the acinar cells; this depolarization could be blocked by atropine, suggesting that there are cholinergic receptors on the acinar cell membrane. The miniature potentials from pancreatic acinar tissue were also blocked by atropine, and potentiated by the anticholinesterase physostigmine, observations which are in accordance with the hypothesis that the miniature potentials are indeed of cholinergic origin.

The miniature potentials in acinar cells differed from the miniature end-plate potentials found at the neuromuscular junction (Fatt & Katz, 1952) in having a longer time course and a larger variability in amplitude. If there is electrical communication between acinar cells through 'tight junctions' of low resistance it is possible that miniature depolarization potentials occurring in adjacent cells may be recorded in an attenuated form from the impaled cell. This may explain the wide distribution of amplitudes for the acinar cell miniature potentials. Alternatively, the wide dispersion in amplitude might be explained by release of quanta at sites quite remote from the cell in which the intracellular recording electrode is located.

The apparent deviation of the observed curve of miniature potential discharge interval from that predicted for a stochastic time series (Fig. 4) may be due to a change in the mean frequency occurring during the prolonged period of observation. Departure from absolute stationarity is, for example, likely over periods exceeding a few minutes for the miniature potentials recorded at muscle end-plates (Gage & Quastel, 1966). Application of the more direct test for conformity to a Poisson process described by Gage & Hubbard (1965) for short time periods revealed that the miniature potential discharge in pancreatic acinar cells is consistent with a Poisson distribution at the $P < 5$–$20\,\%$ level.

Both acetyl β-methylcholine and pancreozymin stimulate zymogen secretion from the pancreas (Hokin, 1968*a*, *b*; Kulka & Sternlicht, 1968; Webster, 1968) and in our experiments both depolarize the acinar cells. Secretin stimulates the pancreas to release HCO_3^- ions probably by acting on the duct cells (Bayliss & Starling, 1902; Becker, 1962) but it had no depolarizing influence on the acinar cells (see Fig. 9). Duct cells are unlikely to have been impaled in the present experiments because of their relatively small size and small number in the pancreas compared with the larger predominantly occurring acinar cells. Unlike the effect of glucose on islet cells (Dean & Matthews, 1970*a*) neither acetyl β-methylcholine nor pancreozymin induced electrical activity in the acinar cells. However, the fact that pancreatic nerve stimulation, parasympathomimetic agents and pancreozymin all induce protein secretion and all were found to depolarize the acinar cells, suggests that pancreatic secretion may be triggered by a

permeability change induced across the acinar cell membrane. The influx of Ca^{2+}, or of Ca^{2+} and Na^{+}, may then be the critical determinant in stimulus-secretion coupling as appears to be the case in other, particle storing, secretory cells (Douglas, 1968).

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